Induction of Pseudopregnancy in the Mongolian Gerbil (Meriones unguiculatus) by Vaginal Stimulation

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Abstract: In rats, pseudopregnancy has been induced by mating with vasectomized males, by mechanical stimulation of the uterine cervix with a glass rod or vibrator, and by stimulation of the vagina with a tampon. On the other hand, no practical data are available in reports on the induction of pseudopregnancy in Mongolian gerbils. Pseudopregnancy of gerbils has been induced by mating with vasectomized males. But this method was uncertain because the incidence of pseudopregnancy was lower than that obtained in rats by other means. In the present study, two experiments were undertaken as follows. 1) Copulatory behavior of gerbils was observed for one hour to determine the most effective stimulation interval. 2) From the results of Experiment 1, female gerbils in estrus were mechanically stimulated to test the effectiveness of inducing pseudopregnancy by vaginal stimulation at various time intervals. The results of these experiments indicated that, although the frequency of copulatory behavior varied among individuals, on average the most effective method for inducing pseudopregnancy was stimulation of 5 min duration and at 20 or 30 min intervals. Because the incidence of pseudopregnancy induced by such mechanical stimulation (83.3%) was higher than that induced by mating with vasectomized males (30.0%), this method might be useful in inducing pseudopregnancy in Mongolian gerbils.

Key words: mechanical stimulation, pseudopregnancy, vasectomized males

Mongolian gerbils (Meriones unguiculatus) have been widely used in various experiments as a laboratory animal [1, 3–5, 7–9] but there have been few reports about the induction of pseudopregnancy in Mongolian gerbils. Pseudopregnancy has been induced by mating of gerbils with vasectomized males but this method was uncertain and the incidence of pseudopregnancy was lower than that obtained in rats by various methods. In rats, pseudopregnancy has been induced by mating with vasectomized males, by mechanical stimulation of the uterine cervix with a glass rod or vibrator, by electrical stimulation of either the uterine cervix and vagina or the head and spinal cord, by hormonal treatment with progesterone, estrogen or prolactin and by stimulation of the vagina with a tampon. The incidence of pseudopregnancy induced by classic mechanical stimulation (glass rod) was generally lower than that obtained after sterile mating but De Feo [2] reported that the induction of pseudopregnancy with a mechanical vibrator appeared to be as effective as sterile mating. Yang [12] reported the induction of pseudopregnancy with a vaginal tampon. After insertion of the tampon for 1–10 min, 69–80%
of estrus and metestrus rats became pseudopregnant, and after insertion of the tampon for 4 hr, 100% of estrus or metestrus rats, 19% of diestrus rats and 46% of proestrus rats became pseudopregnant. Staples [10] electrically stimulated normally cycling female rats at various stages of the estrous cycle to test the effectiveness of a modified probe for the induction of pseudopregnancy. This method was found to be effective throughout vaginal estrus but was much less effective during early and late proestrus or early metestrus. Meyer et al. [6] induced pseudopregnancy in 69% of rats stimulated with a glass rod during Stage 2 or 3 of the estrous cycle, which is during early and late vaginal cornification. Sydnor [11] was able to induce pseudopregnancy with LTH more effectively at the time of ovulation at proestrus.

In this study, two experiments were conducted. In experiment 1, copulatory behavior of gerbils was observed every minute for one hour to determine the most effective stimulation time and the most effective interval of stimuli to be applied in experiment 2. In experiment 2, vaginas were mechanically stimulated at different times of the day and at different intervals to determine the most effective conditions for inducing pseudopregnancy at estrus.

Animals: Mongolian gerbils were obtained from a self-breeding stock in our laboratory. Mature virgin females of 4 months of age were used in this study. Two female gerbils were put in a polycarbonate cage (Sebit Co. Ltd., Tokyo, Japan) bedded with soft chips (Japan SLC Co. Ltd., Shizuoka, Japan) in the conventional manner. The light-dark cycle of the room was maintained with 12 (L): 12 (D), lights on at 6:00 and lights off at 18:00. The gerbils were fed a standard laboratory diet (Fl: Funabashi Farm Co. Ltd., Chiba, Japan), and formula feeds (Tsukuba: Nippon Nosan Kogyo Co. Ltd., Kanagawa, Japan) were given every other day. Fresh water was given ad libitum. Daily vaginal smears were taken from each gerbil at 10:00 for the pre-examination. Mature females, cycling normally for two completed estrous cycles, were used in this study at estrus of the third cycle. In this study, female Mongolian gerbils, which were found to be in the estrus stage in the morning, were injected with gonadotrophic hormone. Mature male Mongolian gerbils of 4 months of age were used for observations of the frequency of copulatory behavior during a one-hour period to determine the optimum time interval between mechanical stimulations. Males were paired with females and the male whose sperms were confirmed in a vaginal smear on Day 1 (the day when sperm were found in the vaginal smear at 10:00 was called Day 1) was employed for observation of copulatory behavior.

Statistical analysis: Statistical significance was measured by chi-square test.

Experiment 1. Observation of copulatory behavior every minute for 1 hr: Four 4-month-old mature virgin female Mongolian gerbils were used for this study (numbered 1–4). Vaginal smears of these animals taken in the mornings showed their estrus stage. Each of the females was made to pair with indicated males (3–4 months of age) at 17:00 and the copulatory behavior was observed every minute of the following hour to determine the most effective stimulation time interval for experiment 2. The frequency of copulatory behavior of gerbils was regarded as the number of mountings of the female by the male. An observation time of 1 hr was selected because we had previously found that the maximum frequency of copulatory behavior occurs during this period [8].

Experiment 2. Induction of pseudopregnancy: Thirty-four mature virgin female Mongolian gerbils (3 months of age) were used in this study. Daily vaginal smears were taken from each animal for the duration of the experimental period. Females, cycling normally for two complete estrous cycles, were mechanically stimulated at the estrus stage of the third cycle. Vasectomized males were used in this study as a control. The vibrator is shown in Fig. 1. The vibrator (Fig. 1) rotates at 83 rpm. For stimulation, females were fixed to a corkboard and then stimulated with the vibrator. Mechanical stimulation was given to the external uterine orifice and vagina with the vibrator. The experiment was conducted with five groups of gerbils (control and groups A–D), each receiving a different treatment: 1) as control, in order to induce pseudopregnancy, ten females were mated with vasectomized males. 2) as Group A, six females were subjected to 5 min of stimulation starting at 16:00, and then a 20 min pause. This cycle was repeated three times. 3) as Group B, six females were subjected to 5 min of stimulation starting at 16:00, and then a 30 min pause. This cycle was repeated three times. 4) as Group C, six females were subjected to 5 min of stimulation starting at 17:00, and then a 20 min pause. This cycle was repeated three
times. 5) as Group D, six females were subjected to 5 min of stimulation starting at 17:00, and then a 30 min pause. This cycle was repeated three times. The beginning time of stimulation, 16:00 or 17:00 was based on the results of our previous study [8]. Pseudopregnancy was defined as a prolonged diestrus with a delay of more than 12 days until the following estrus.

In experiment 1, frequency of copulatory behavior was found to vary among the females (Fig. 2). In this result, the observed copulatory behavior was found to vary among the females. Copulatory behavior occurred intermittently; female No. 1 showed three active phases from 4 to 12 min, 17 to 27 min and 55 to 60 min. Female No. 2 showed two active phases from 27 to 35 min and 46 to 55 min. Female No. 3 showed two active phases from 1 to 5 min and 31 to 39 min. Female No. 4 showed three phases from 1 to 6 min, 35 to 39 min and 56 to 59 min (Fig. 2).

From these results, we found that the copulatory be-

![Image](image_url)

**Fig. 1.** This photograph is the equipment used to induce pseudopregnancy in Mongolian gerbils (*Meriones unguiculatus*). R is the rod. The rod is inserted into the vagina and rotates. B is the button. R rotates when B is pushed. M is the motor.

**Fig. 2.** Frequency of copulatory behavior per minutes in the Mongolian gerbil.
Behavior of female gerbils continued for an average of 5 min and that the interval between mountings of gerbils was 20 or 30 min. Therefore, in Experiment 2, we used a stimulation time of 5 min and an interval of 20 or 30 min. The females were stimulated three time to increase the probability that pseudopregnancy was induced.

The results of experiment 2 are shown in Table 1. In the control, 7 of 10 females mated with vasectomy males, but in only 3 of these 7 females (42.9%) was pseudopregnancy induced. In Group A, six females were stimulated with a vibrator at 16:00 and then after a 20 min pause, in 3 of 6 females (50.0%) was pseudopregnancy induced. In Group B, six females were stimulated with a vibrator at 16:00 and then after a 30 min pause, in 4 of 6 females (66.7%) was pseudopregnancy induced. In Groups C and D, 6 females were stimulated with a vibrator at 17:00, and then a 20 or 30 min pause, and pseudopregnancy was induced in five (83.3%) of the 6 females. The rates of pseudopregnancy in the two groups were significantly higher than that in the control and Groups A and B (p<0.05). These findings suggested that the experimental induction of pseudopregnancy of gerbils by mechanical stimulus with a vibrator of the uterine cervix and vagina was quite successful when starting 17:00, with a 20 or 30 min interval.

In this study, female Mongolian gerbils which were found to be in the estrus stage in the morning were injected with gonadotrophin hormone. In rats, De Feo [2] reported that the induction of pseudopregnancy with a mechanical vibrator appeared to be as effective as sterile mating. Yang [12] reported the induction of pseudopregnancy with vaginal tampon. He suggested that after insertion of the tampon for 1–10 min, 69–80% of the rats in estrus and metestrus became pseudopregnant, on the other hand, after insertion of the tampon for 4 hr, 100% of estrus or metestrus rats, 19% of diestrus rats and 46% of proestrus rats became pseudopregnant. Staples [10] electrically stimulated normally cycling female rats at various stages of the estrous cycle to test the effectiveness of a modified probe for the induction of pseudopregnancy. This method was found to be effective throughout vaginal estrus but was much less effective during early and late proestrus or early metestrus. Meyer et al. [6] induced pseudopregnancy in 69% of rats stimulated with a glass rod during Long and Evans Stage 2 or 3 of the estrous cycle, which is during early and late vaginal cornification.

### Table 1. The ratio of the induction of pseudopregnancy in the Mongolian gerbils

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of Animals</th>
<th>Number of the pseudopregnancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>3 (30.0)%</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>3 (50.0)%</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>4 (66.7)%</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>5 (83.3)%</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>5 (83.3)%</td>
</tr>
</tbody>
</table>

* Females were mated with vasectomy males (control). Stimulation was begun at 16:00, and then there was a pause for 20 or 30 min (A, B). Stimulation was begun at 17:00, and then there was a pause for 20 or 30 min (C, D). ^: the same column with different superscript is significantly different (p<0.05).

In this report, female Mongolian gerbils were stimulated at various times. The incidence of pseudopregnancy induced by mechanical stimulation was higher than that obtained with vasectomy males that were mated with the females. Especially when the Mongolian gerbils were given the stimulation at 17:00, pseudopregnancies were induced in 83.3% of the females. In this study, the rate of pseudopregnancy in the Mongolian gerbils matched the rate of pseudopregnancy in the rats. In conclusion, the present study demonstrated that stimulation of the cervix and vagina with a vibrator was a useful experimental condition for induction of pseudopregnancy in Mongolian gerbils.

### References